Am. J. Hum. Genet. 49:1082–1090, 1991

Carbonic Anhydrase II Deficiency Syndrome in a Belgian Family Is Caused by a Point Mutation at an Invariant Histidine Residue (107 His→Tyr): Complete Structure of the Normal Human CA II Gene

Patrick J. Venta,*,† Rosalind J. Welty,* Thomas M. Johnson,* William S. Sly,‡ and Richard E. Tashian*

*Department of Human Genetics, University of Michigan School of Medicine, Ann Arbor; †Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing; and ‡Department of Biochemistry, St. Louis University School of Medicine, St. Louis

Summary

Carbonic anhydrase II (CA II), which has the highest turnover number and widest tissue distribution of any of the seven CA isozymes known in humans, is absent from the red blood cells and probably from other tissues of patients with CA II deficiency syndrome. We have sequenced the CA II gene in a patient from a consanguinous marriage in a Belgian family and identified the mutation that is probably the cause of the CA II deficiency in that family. The change is a C-to-T transition which results in the substitution of Tyr (TAT) for His (CAT) at position 107. This histidine is invariant in all amniotic CA isozymes sequenced to date, as well as the CAs from elasmobranch and algal sources and in a viral CA-related protein. His-107 appears to have a stabilizing function in the structure of all CA molecules, and its substitution by Tyr apparently disrupts the critical hydrogen bonding of His-107 to two other similarly invariant residues, Glu-117 and Tyr-194, resulting in an unstable CA II molecule. We have also completed the intron-exon structure of the normal human CA II gene, which has allowed us to prepare PCR primers for all exons. These primers will facilitate the determination of the mutations in other inherited CA II deficiencies.

Introduction

Of the three inherited forms of osteopetrosis that are known to occur in humans, only one has been shown to be associated with renal tubular acidosis, cerebral calcification, and the absence of carbonic anhydrase II (CA II) in patients homozygous for the enzyme deficiency (Sly et al. 1983, 1985*b*; Sly 1989). To date, more than 30 cases of this disorder, termed "carbonic anhydrase II deficiency syndrome," have been diagnosed from a variety of ethnic backgrounds, including Italian, German, French, Hispanic, Afro-American, and Arabian (for reviews, see Cochat et al. 1987; Sly 1989). Because of the absence of CA II in erythrocytes, kidney, and probably in other tissues as well (Sly et al. 1983, 1985*a*; Krupin et al. 1985; Sato et al. 1990), the most likely cause of this disorder would be expected to be a mutation in the regulation or structure of the CA II gene. Toward this end, we have sequenced the CA II gene from a patient with CA II deficiency syndrome in a Belgian family first described by Vainsel et al. (1972).

Here, we provide evidence that an amino acid substitution at an invariant site in the CA II molecule is the mutation responsible for the CA II deficiency in the Belgian family. Also, in order that other deficiency mutations at this locus may be readily detected, PCR primer sets are given. In addition, we describe for the first time the complete structure of the human CA II gene.

Received May 28, 1991; revision received July 18, 1991.

Address for correspondence and reprints: Richard E. Tashian, Department of Human Genetics, M4708 Medical Science II, University of Michigan School of Medicine, Ann Arbor, MI 48109–0618.

0002-9297/91/4905-0019$02.00

Material and Methods

Genomic Cloning

DNA was isolated by standard methods (Maniatis et al. 1982) from the white cells saved from the blood typing of the Belgian patient who had previously been diagnosed with CA II deficiency syndrome (proband in family IV in fig. 1 of Sly et al. 1985*b*). The DNA was partially digested with *Sau*3AI and cloned into the *Bam*HI site of EMBL3 (Frischauf et al. 1983). The DNA from a patient from Kuwait (male proband in family V in fig. 1 in Sly et al. 1985*b*) who had CA II deficiency syndrome was cloned in the same way. The two libraries, each containing 1 million independently derived recombinant phage, were screened for CA II–containing sequences by radiolabeled human CA II cDNA by using standard techniques (Maniatis et al. 1982). Positive clones were plaque purified, and fragments of the inserts were subcloned into pBSM13- (Stratagene, San Diego) by using a variety of restriction endonucleases.

Sequence Analysis

Regions containing exons were determined using a combination of (*a*) Southern blot analysis of the clones by the cDNA probe and (*b*) restriction-site data found in the cDNA. The previously determined structure of the mouse CA II gene (Venta et al. 1985*b*) also served as a guide for locating the exons. The location of the first two exons had previously been determined (Venta et al. 1985*a*). Sequencing was performed on the double-stranded plasmids (Hattori and Sakaki 1986) by using Sequenase (United States Biochemical Corp.) and later by direct sequence analysis of the double-stranded PCR products (Bachmann et al. 1990).

In Vitro Mutagenesis

The human CA II cDNA (Montgomery et al. 1987) was subcloned into the bacterial expression vector pKK233-2 (Amann and Brosius 1985) by filling in both the *Tth*111I site overlaying the initiation codon and the *Nco*I site in the vector, followed by blunt-end ligation. The 3′ end of the cDNA was ligated into the expression vector by using the unique *Hin*dIII sites contained in the vector and the 3′ untranslated region of the cDNA. The mutation was made in the original cDNA clone by using a modified PCR-based method (Ho et al. 1989) and then was transferred into the CA II expression vector by replacing the *Pst*I-*Bam*HI fragment. This region was sequenced to confirm that only the one nucleotide had been changed—and that it was therefore the only difference between the normal and mutant CA II expression plasmids.

Expression and Western Blotting

The normal and mutated CA II cDNA expression vectors were transformed into the *Escherichia coli* host Y1089 (Young and Davis 1983), which previously had been cured by pMC9 by acridine orange (Miller 1972). The bacteria were grown to confluence in LB broth and were lysed by addition of lysozyme and several cycles of freeze-thawing, and the lysate was cleared by centrifugation. Protein concentrations of the supernatants were determined using a dye-binding assay (Bradford 1976), and 50 µg of protein from each lysate were run on an SDS gel and were Western-blotted using a standard technique (Towbin et al. 1979). The blots were tested for CA II immunoreactivity by using rabbit anti-human CA II antiserum and goat anti-rabbit IgG-alkaline phosphatase conjugate, followed by staining for phosphatase activity (Shapiro et al. 1989).

Results

Structure of the Human CA II Gene

The clones containing the human CA II gene are shown in figure 1. The H25 clone was previously isolated from a library (Lawn et al. 1978) containing a presumably normal CA II gene (Venta et al. 1985*a*). The H19.3 clone is from another normal library prepared in our laboratory (R. J. Welty and P. J. Venta, unpublished data). The 8a clone was derived from the Belgian patient, and the Kuw 8.1, 7.2, and 6.3 clones were isolated from the Kuwaiti patient. Other clones were also derived from these libraries but were not used in the determination of the structure of the gene.

The restriction map of the clones agrees exactly with data derived from Southern blots of normal human DNA probed with the human CA II cDNA (Venta et al. 1987; data not shown), except that an inversion occurred within about 1 kb of each end of clone 8a. This inversion is a cloning artifact and not indicative of the structure of the gene in the uncloned DNA (see Venta et al. 1987). The precise inversion points contained within this clone have not been determined.

Various subclones containing the different exons were sequenced as described in Material and Methods. The sequence containing exons 1 and 2, intron 1, and about 500 bp of 5′ flanking region have been reported elsewhere (Venta et al. 1985*a;* Shapiro et al. 1987). This region contains the minimal human CA II promoter (Shapiro et al. 1987). In the present study, we have extended this sequence to the first *Eco*RI site upstream of the promoter. This sequence and the sequences surrounding the other exons have been submitted to GenBank. The PCR primers used to amplify and sequence the exons of the human CA II gene are

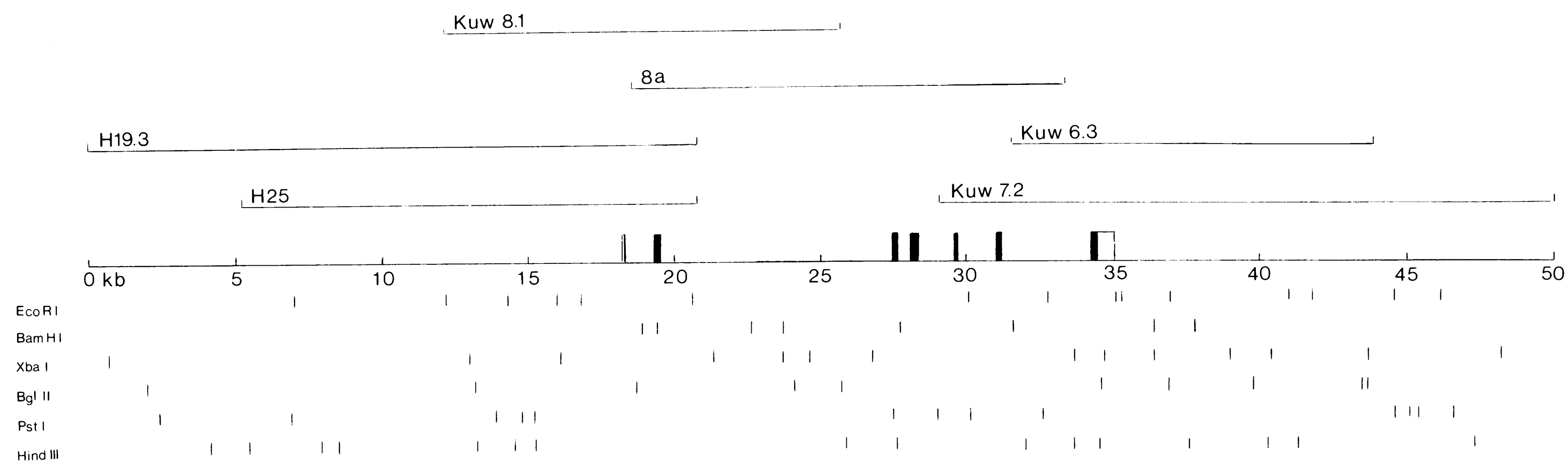

Figure 1 Structure of human carbonic anhydrase II gene. The black boxes and white boxes indicate the positions of coding regions and untranslated regions, respectively. The inserts of the clones used to determine the structure of the gene are mapped above the exons and are described more fully in the text. The restriction-endonuclease map is given on the lower part of the figure.

Table 1

PCR Primers Used to Amplify and Sequence Exons

Exon	Primer 1[a]	Primer 2[a]	Size[b] (bp)
1........	CCCCCGAGCACGAAGTTG	GGCCGCGGTAAACAGCATGT	283
2........	TGGAACTGAGCAGATTGGCT	GGAAAACAGCTACTGGCTAA	346
3........	TCTGGATTGAATTTTCAGAG	CACTGGGCGTGAGTTGAATT	407
4........	ATTGAATAAAATCTGTCAGC	CCTAAGCTCTTTTGTAGAATG	259
5........	TATGGTGTCAGTGTCATCAAG	AATGGAGTATTGAAATTGGTACC	283
6........	TTGTGTCTGCTGCTCTCCTA	TCAGGTCTCTCTCACTCTGG	319
7........	GATTACAGCATGAGCCACTGC	AGGGTCCAAATCACCAAGGT	323

[a] The 5′ end is on the left. Some of the primers had artificial linkers attached (six or seven extra nucleotides) that are not necessary for amplification and are not shown.
[b] For primers without linkers.

shown in table 1. The sequences of the exonic regions agreed exactly with the previously published cDNA sequences (Montgomery et al. 1987; Murakami et al. 1987; Forsman et al. 1988). The *Bst*NI polymorphic site at amino acid position 189 (Venta and Tashian, in press) is contained in exon 6 of the Kuwaiti gene but not in the Belgian gene. All splice site sequences (table 2) conformed to the consensus rules determined elsewhere (Mount 1982).

Determination of the Mutation

The mutation was found in exon 3 and converts the normal histidine 107 residue into a tyrosine residue (fig. 2). The mutation produces an *Acc*I site which can be conveniently typed by PCR and digestion with the restriction enzyme (see fig. 3).

Western Blot Analysis

The presence of the mutation in the mutant expression plasmid was confirmed by *Acc*I digestion (fig. 3) and direct sequence analysis (data not shown). Expression of the mutant CA II cDNA in *Escherichia coli* was undetectable (fig. 4). A band of CA II immunoreactivity was present in extracts prepared from *E. coli* containing the expression plasmid with the normal CA II cDNA.

Discussion

The CA gene family in mammals is now known to encode at least eight CA or CA-like isozymes, termed "CA I"–"CA VII," "CA Y" (= CA V?), and "CARP" (Amor-Gueret and Levi-Strauss 1990; Kato 1990; Hewett-Emmett and Tashian 1991). Of these, CA II displays the highest specific activity and is expressed in the widest variety of tissues, where it functions in such important processes as the formation of pancreatic juice, cerebral spinal fluid, saliva, aqueous humor, and gastric juice; bicarbonate reabsorption (kidney); and bone resorption (see Tashian 1989). Despite the

Table 2

Nucleotide Sequences of Donor and Acceptor Splice Sites of Human CA II Gene

Donor[a]	Intron (size)	Acceptor[a]
ACAACGgtgagtgccggcg	1 (1.2 kb)	ttctttcccccagGACCTG
AAGCAGgtcagtgtttaga	2 (7.2 kb)	gttttaattttagGTGCTC
GCAGAAgtaagatatactt	3 (.7 kb)	tcttcggccttagCTTCAC
TTGAAGgttagttgatgac	4 (1.3 kb)	tgtttttctttagGTTGGC
ACAAAGgtaaatttgaatt	5 (1.5 kb)	tgccttgttctagGGCAAG
GAGCAGgtttgttttgtaa	6 (3.1 kb)	ttgtgrcttttagGTGTTG
C g AGgt agt .... A a	Concensus	ccccccccc c n agG ttttttttt t

[a] Capital letters represent exonic regions, and lowercase letters represent intronic regions. The concensus sequences are from Mount (1982).

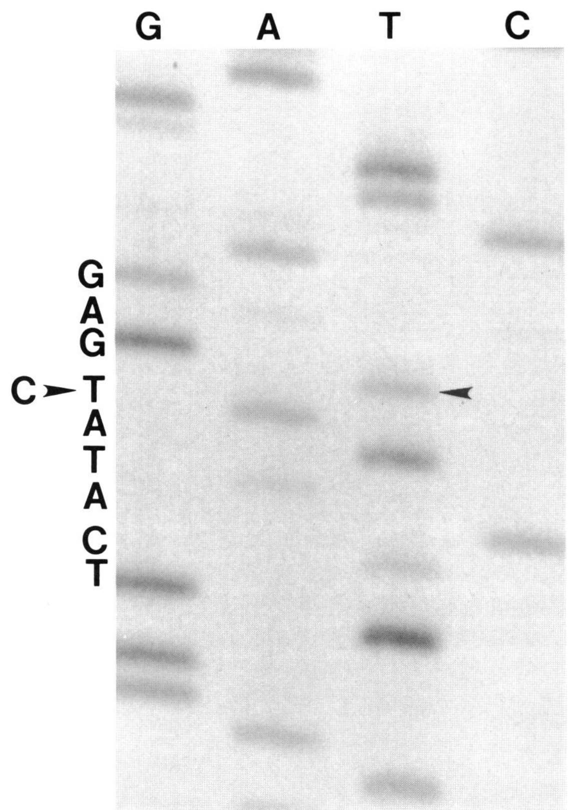

Figure 2 Sequencing gel showing deficiency causing nucleotide substitution contained in exon 3. The C-to-T transition that causes the His-to-Tyr substitution is marked by an arrow on the sequencing gel. Note that the substitution creates an *Acc*I restriction site (GTATAC).

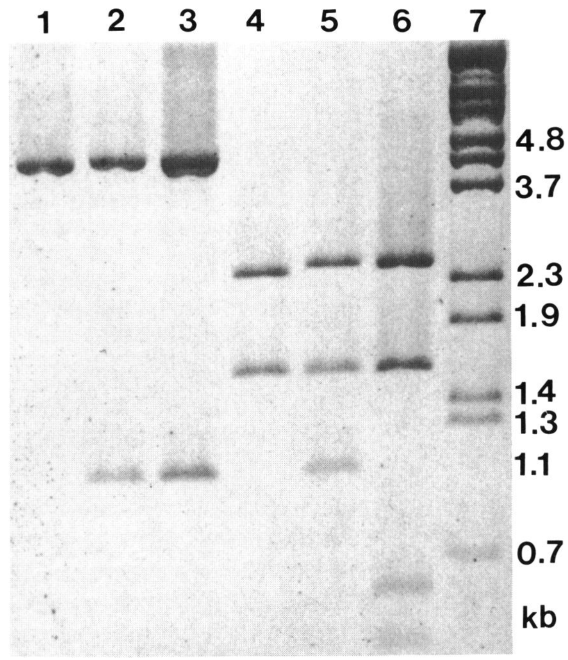

Figure 3 Detection of the mutation by using *Acc*I. The figure is a reverse image of an ethidium bromide–stained gel. Lanes 1–3 include plasmids cut with *Eco*RI and *Hin*dIII, and lanes 4–6 include plasmids cut with both of these two enzymes and with *Acc*I. Lanes 1 and 4, pKK233-2 expression vector. Lanes 2 and 5, pKKHCAII (His 107). Lanes 3 and 6, pKKHCAII (Tyr 107). Lane 7, *Bst*NI-digested lambda phage DNA size markers. Note that the wild-type cDNA band (1.1 kb; lane 5) is not cut with *Acc*I, while the mutant cDNA (lane 6) is cut with *Acc*I into two smaller bands. This mutation can easily be typed in affected individuals by using the PCR primers for exon 3 and *Acc*I (table 1). The supply of DNA from this individual was exhausted before the mutation had been identified and thus could not be directly shown by this method.

wide distribution of CA II and its participation in many physiological processes, clinical manifestations (i.e., osteopetrosis and renal tubular acidosis) only occur by the absence of CA II in osteoclasts (bone resorption) and renal tubular cells (bicarbonate reabsorption). The lack of clinical effects in other tissues may be partly explained by the presence of other CA isozymes (e.g., CA I, CA III, and CA IV) that are normally expressed with CA II in these other tissues and that can fulfill its function. For example, it was clearly demonstrated that the red cell CA I can effectively carry out the important role of CA in CA II–deficient red cells (Dodgson et al. 1988).

The CA II Deficiency Mutation

We have found a mutation in exon 3 of the human CA II gene in a patient with CA II deficiency syndrome. This mutation converts a histidine residue (CAT) at position 107 to tyrosine (TAT). Because the patient is the offspring of a consanguineous marriage, and because deficiency alleles of CA II are rare, we can assume that both alleles are identical by descent and that a second allele does not have to be identified.

The mutation substitutes tyrosine for a histidine residue that is invariant at position 107 in all evolutionarily related CAs sequenced to date, including all of the amniotic CA isozymes (see Amor-Gueret and Levi-Strauss 1990; Kato 1990; Hewett-Emmett and Tashian 1991), a red cell CA from tiger shark (Bergenhem and Carlsson 1990), a periplasmic CA from the green alga *Chlamydomonas rheinhardtii* (Fukuzawa et al. 1990), and a CA-like transmembrane protein from vaccinia virus (Niles and Seto 1988). Because the conserved histidine (His-107) is located in the active site of human CA I, human CA II, and bovine CA III (see Notstrand et al. 1974; Ericksson 1988), as determined by crystallographic analysis, it was of interest to examine the position effect of Tyr-107 in the

Figure 4 Western blot showing destabilizing effect of substitution. The arrows indicate the position of the 29-kb band of CA II immunoreactivity from *Escherichia coli* lysates containing various plasmids. Lane 1, Molecular weight markers, including rabbit CA II. Lane 2, Human CA II, purified from blood. Lane 3, Lysate from *E. coli* containing no plasmid. Lane 4, *E. coli* containing expression vector (pKK233-2) with no insert. Lane 5, *E. coli* containing expression vector with normal human CA II cDNA. Lane 6, *E. coli* containing expression vector with mutated human CA II cDNA. Immunoreactivity is seen with the normal CA II cDNA (His 107; lane 5) but not with the mutated cDNA (Tyr 107; lane 6).

active site by using a computer molecular-graphic-modeling program. The results of this analysis showed that the His→Tyr substitution produces a distortion in the active-site cavity (A. Liljas, personal communication). As depicted in figure 5, His-107 is normally hydrogen bonded to Glu-117 and Tyr-194, bonding to both of which would be disrupted by the mutation.

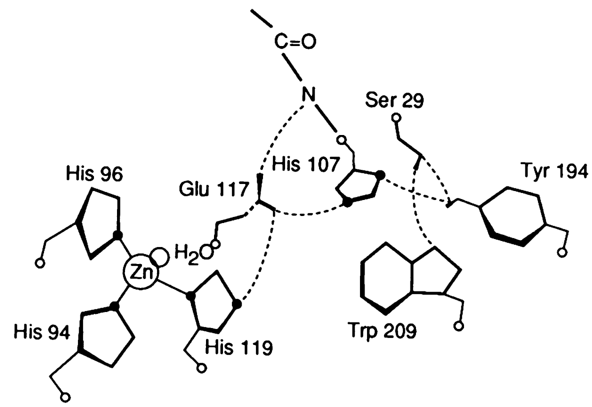

Figure 5 Partial representation of hydrogen bonding network in active site of human carbonic anhydrase II. The figure is modified from fig. 3 in Kannan et al. (1975) or fig. 7 in Notstrand et al. (1974).

Evidence that His-107 is structurally important is further indicated by its position in aromatic cluster III, as determined by crystallographic analysis (Notstrand et al. 1974). The fact that His-107 is strongly hydrogen bonded to Tyr-194 and Glu-117 suggests that it may be involved in a charge distribution pattern involving the zinc ion; however, as suggested by Notstrand et al. (1974), its primary role may be to stabilize the molecule in this region. This mutation has thus provided us with information which supports the fact that His-107 has an important structural role in the CA molecule, a role first indicated by three-dimensional analysis and now verified by a mutational event.

We believe that the instability of the mutant molecule in *Escherichia coli* is indicative of the instability in the affected human cells. Although we were unable to detect any of the mutant CA II in the *E. coli* extracts, it is possible that small amounts of residual activity remain in human tissues other than the red blood cells. Such residual activity might explain why the clinical symptoms of this patient are somewhat milder than those of other patients with this disorder. In particular, the intelligence of the Belgian patient appears to be normal, whereas most patients with this syndrome have had evidence of mental retardation (see Sly et al. 1985*b*).

It had been our hope that, if some mutant CA II could be detected in *E. coli,* we would be able by biochemical methods to purify the protein and directly demonstrate instability. Although we have not been able either to demonstrate the instability of this protein in vitro or to demonstrate a decreased level of CA II in the osteoclasts or kidneys, it seems highly unlikely that this mutation is not the cause of the disease in this patient.

Because of the relatively wide ethnic distribution of the CA II deficiency syndrome, and because of the variability observed in some of the symptoms (e.g., mental retardation, renal tubular acidosis, and cerebral calcification), it is possible that several different mutations account for the clinical heterogeneity reported in patients with the syndrome. Defining the mutations resulting in CA II deficiency in these pedigrees will address this question. It is of interest that a mouse model for CA II deficiency has been reported that does not show the bone or brain manifestations of this syndrome (Lewis et al. 1988). Although CA II is absent in all mouse tissues examined, no osteopetrosis or cerebral calcification was detected, even in the longest-lived (20 mo) mice that were examined. There is, however, definite growth retardation and renal tubular acidosis, and Spicer et al. (1988) have reported vascular calcification in arterial capillaries.

Structure of the Human CA II Gene (CA2)

Of the eight mammalian CA genes that are known, four have been characterized. These are (1) the CA I genes of human (Brady et al. 1989), mouse (Fraser et al. 1989), and pigtail macaque (Nicewander 1990); (2) the CA II gene of mouse (Venta et al. 1985*b*); (3) the CA III gene of human (Lloyd et al. 1987); and (4) the CA VII gene of human (Montgomery et al., in press). All of these genes have seven exons which are interrupted in the same positions by six introns, and they range in size from about 10 kb for human CA VII to about 50 kb for human CA I (see Hewett-Emmett and Tashian 1991). The CA I gene differs in having an additional upstream noncoding exon (exon 1a) which is separated (in humans) from exon 1 by a 37-kb intron (Brady et al. 1989; Fraser et al. 1989).

The structure of the human CA II gene which is located at q22 on chromosome 8 (Nakai et al. 1987) is very similar to the previously published structures of the mouse and chicken CA II genes (Venta et al. 1985*b;* Yoshihara et al. 1987) and has the same splicing points in the coding regions as do all the other CA genes determined thus far (see Hewett-Emmett and Tashian 1991). The sequence information was used to develop PCR primers to all of the coding regions of the exons as well as to all splice junctions and will be useful in determining the molecular defects found in other CA II deficiency alleles.

Note added in proof: GenBank accession numbers for the human CA II gene sequences are M77176–M77181.

Acknowledgments

This work was supported by NIH grants GM 24682 (to R.E.T.) and GM 34182 and AI 2235 (to W.S.S.). The blood samples from the Belgian patient were sent to us by Dr. Marc Vainsel (Hôpital Universitaire Saint-Pierre, Brussels). We thank Ya-Shiou L. Yu for her excellent research assistance, and we thank Judy Worley for her welcome help in preparing the manuscript.

References

Amann E, Brosius J (1985) "ATG vectors" for regulated high-level expression of cloned genes in *Escherichia coli.* Gene 40:183–190

Amor-Gueret M, Levi-Strauss M (1990) Nucleotide and derived amino-acid sequence of a cDNA encoding a new mouse carbonic anhydrase. Nucleic Acids Res 18:1646

Bachmann B, Lüke W, Hunsmann G (1990) Improvement of PCR amplified DNA sequencing with the aid of detergents. Nucleic Acids Res 18:1309

Bergenhem N, Carlsson U (1990) Partial amino acid sequence of erythrocyte carbonic anhydrase from tiger shark. Comp Biochem Physiol [B] 95:205–213

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Brady HJM, Sowden JC, Edwards M, Lowe N, Butterworth PHW (1989) Multiple GF-1 binding sites flank the erythroid specific transcription unit of the human carbonic anhydrase I gene. FEBS Lett 257:451–456

Cochat P, Loras-Duclaux I, Guibaud P (1987) Déficit en anhydrase carbonique II: ostéopétrose, acidose rénale tubulaire et calcifications intrâcraniennes: revue de la littérature à partir de trois observations. Pediatrie 42:121–128

Dodgson SJ, Forster RE, Sly WS, Tashian RE (1988) Carbonic anhydrase activity of intact CA II–deficient erythrocytes. J Appl Physiol 65:1472–1480

Ericksson AE (1988) Structural differences between high and low activity forms of carbonic anhydrase. PhD thesis, University of Uppsala, Uppsala

Forsman C, Behravan G, Osterman A, Jonsson B-H (1988) Production of active human carbonic anhydrase II in *E. coli.* Acta Chem Scand [B] 42:314–318

Fraser P, Cummings P, Curtis P (1989) The mouse carbonic anhydrase I gene contains two tissue-specific promoters. Mol Cell Biol 9:3308–3313

Frischauf A-M, Lehrach H, Poustka A, Murray N (1983) Lambda replacement vectors carrying polylinker sequences. J Mol Biol 170:827–842

Fukuzawa H, Fujiwara S, Yamamoto Y, Dioniso-Sese M, Miyachi S (1990) cDNA cloning, sequence, and expression of carbonic anhydrase in *Chlamydomonas reinhardtii:* regulation by environmental CO_2 concentration. Proc Natl Acad Sci USA 87:4383–4387

Hattori M, Sakaki Y (1986) Dideoxy sequencing method using denatured plasmid templates. Anal Biochem 152: 232–238

Hewett-Emmett D, Tashian RE (1991) Structure and evolutionary origins of the carbonic anhydrase multigene family. In: Dodgson SJ, Tashian RE, Gros G, Carter ND (eds) The carbonic anhydrases: cellular physiology and molecular genetics. Plenum, New York, pp 15–32

Ho SN, Hunt HD, Horton RM, Pullen JK, Pease LR (1989) Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene 77:51–59

Kannan KK, Notstrand B, Fridborg K, Lövgren S, Ohlsson A, Petef M (1975) Crystal structure of human erythrocyte carbonic anhydrase B: three dimensional structure at a nominal 2.2-Å resolution. Proc Natl Acad Sci USA 72: 51–55

Kato K (1990) Sequence of a novel carbonic anhydrase–related polypeptide and its exclusive presence in Purkinje cells. FEBS Lett 271:137–140

Krupin T, Sly WS, Whyte MP, Dodgson SJ (1985) Failure of acetazolamide to decrease interocular pressure in patients with CA II deficiency. Am J Ophthamol 99:396–399

Lawn RM, Fritsch EF, Parker RC, Blake G, Maniatis T (1978) The isolation and characterization of linked α- and β-globin genes from a cloned library of human DNA. Cell 15:1157–1174

Lewis SE, Erickson RP, Barnett LB, Venta PJ, Tashian RE (1988) N-ethyl-N-nitrosourea-induced null mutation at the mouse *Car-2* locus: an animal model for human carbonic anhydrase II deficiency syndrome. Proc Natl Acad Sci USA 85:1962–1966

Lloyd J, Brownson C, Tweedie S, Charlton J, Edwards YH (1987) Human muscle carbonic anhydrase: gene structure and DNA methylation patterns in fetal and adult tissues. Genes Dev 1:594–602

Maniatis T, Fritsch EF, Sambrook J (1982) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY

Montgomery JC, Venta PJ, Eddy RL, Fukushima Y-S, Shows TB, Tashian RE. Characterization of the human gene for a newly discovered carbonic anhydrase, CA VII, and its localization to chromosome 16. Genomics (in press)

Montgomery JC, Venta PJ, Tashian RE, Hewett-Emmett D (1987) Nucleotide sequence of human liver carbonic anhydrase II cDNA. Nucleic Acids Res 15:4687

Mount SM (1982) A catalog of splice junction sequences. Nucleic Acids Res 10:459–472

Murakami H, Marelich GP, Grubb JH, Kyle JW, Sly WS (1987) Cloning, expression, and sequence homologies of cDNA for human carbonic anhydrase II. Genomics 1: 159–166

Nakai H, Byers MG, Venta PJ, Tashian RE, Shows TB (1987) The gene for human carbonic anhydrase II (*CA2*) is located on chromosome 8q22. Cytogenet Cell Genet 44:234–235

Nicewander PH (1990) Sequence and organization of a *Macaca nemestrina* carbonic anhydrase I gene. PhD thesis, University of Michigan, Ann Arbor

Niles EG, Seto J (1988) Vaccinia virus gene D8 encodes a virion transmembrane protein. J Virol 62:3772–3778

Notstrand B, Vaara I, Kannan KK (1974) Structural relationship of human erythrocyte carbonic anhydrase isozymes B and C. In: Markert CL (ed) Isozymes: molecular structure, vol 1. Academic Press, New York, pp 575–599

Sato S, Zhu XL, Sly WS (1990) Carbonic anhydrase isozymes IV and II in urinary membranes from carbonic anhydrase II–deficient patients. Proc Natl Acad Sci USA 87:6073–6076

Shapiro LH, Venta PJ, Tashian RE (1987) Molecular analysis of G + C-rich upstream sequences regulating transcription of human carbonic anhydrase II gene. Mol Cell Biol 7:4589–4593

Shapiro LH, Venta PJ, Yu Y-S, Tashian RE (1989) Carbonic anhydrase II is induced in HL-60 cells by 1,25-dihydroxyvitamin D_3: a model for osteoclast gene regulation. FEBS Lett 249:307–310

Sly WS (1989) The carbonic anhydrase II deficiency syndrome: osteopetrosis with renal tubular acidosis and cerebral calcification. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease, 6th ed, vol 2. McGraw-Hill, New York, pp 2857–2866

Sly WS, Hewett-Emmett D, Whyte MP, Yu Y-SL, Tashian RE (1983) Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. Proc Natl Acad Sci USA 80:2752–2756

Sly WS, Whyte MP, Krupin T, Sundaram V (1985*a*) Positive renal response to intravenous acetazolamide in patients with carbonic anhydrase II deficiency. Pediatr Res 19: 1033–1038

Sly WS, Whyte MP, Sundaram V, Tashian RE, Hewett-Emmett D, Guibaud P, Vainsel M, et al (1985*b*) Carbonic anhydrase II deficiency in 12 families with the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. N Engl J Med 313: 139–145

Spicer SS, Lewis SL, Tashian RE, Schulte BA (1988) Mice carrying a Car-2 null allele lack carbonic anhydrase II immunohistochemically and show vascular calcification. Am J Pathol 134:947–954

Tashian RE (1989) The carbonic anhydrases: widening perspectives on their evolution, expression and function. BioEssays 10:186–192

Towbin H, Staehlin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76:4350–4354

Vainsel M, Fondu P, Cadranel S, Rochmans C, Gepts W (1972) Osteopetrosis associated with proximal and distal tubular acidosis. Acta Pediatr Scand 61:429–434

Venta PJ, Montgomery JC, Hewett-Emmett D, Tashian RE (1985*a*) Comparison of the 5′ regions of human and mouse carbonic anhydrase II genes and identification of possible regulatory elements. Biochim Biophys Acta 826: 195–201

Venta PJ, Montgomery JC, Hewett-Emmett D, Wiebauer K, Tashian RE (1985*b*) Structure and exon to protein domain relationships of the mouse carbonic anhydrase II gene. J Biol Chem 260:12130–12135

Venta PJ, Montgomery JC, Tashian RE (1987) Molecular genetics of carbonic anhydrase isozymes. In: Ratazzi MC, Scandalios JG, Whitt GS (eds) Isozymes: current topics in biological and medical research, vol 14. Alan R Liss, New York, pp 59–72

Venta PJ, Tashian RE. PCR detection of a *Bst*NI polymorphism in exon 6 of the human carbonic anhydrase II locus, *CA2*. Nucleic Acids Res (in press)

Yoshihara CM, Lee JD, Dodgson JB (1987) The chicken carbonic anhydrase II gene: evidence for a recent shift in intron position. Nucleic Acids Res 15:753–770

Young RA, Davis RW (1983) Yeast RNA polymerase II genes: isolation with antibody probes. Science 222:778–782